Oligonucleotide Properties Calculator

Enter Oligonucleotide Sequence Below
OD and Molecular Weight calculations are for single-stranded DNA or RNA

Nucleotide base codes

GAG TTC CTC GGC TC

Reverse Complement Strand(5' to 3') is:

GAG CCG AGG AAC TC

Number of Fluorescent tags per strand:

0 6-FAM 0 TET 0 HEX 0 TAMRA DNA

Minimum base pairs required for single primer self-dimerization: 5

Minimum base pairs required for a hairpin : 4

Calculate | SWAP STRANDS | BLAST2 | Check Self-Complementarity

Physical Constants

Length: 14 bases

GC content: 64 %

Molecular Weight: 4371.8 4

1 ml of a sol'n with an Absorbance of 1 at 260 nm

is 7.571 microMolar 5 and contains 33.1 micrograms.

Melting Temperature (T_M) Calculations

1 43 °C (Basic)

2 50 °C (Salt Adjusted)

3 42 °C (Nearest Neighbor)

50 nM Primer

50 mM Salt (Na^+)

Thermodynamic Constants
Conditions: 1 M NaCl at 25°C at pH 7.

RlogK 33.404 cal/(°K*mol)

deltaH 116.5 Kcal/mol

deltaG 17.5 Kcal/mol

deltaS 302.8 cal/(°K*mol)

To use this calculator, you must be using Netscape 3.0 or later
or Internet Explorer version 3.0 or later, or another Javascript-capable browser
Self-Complementarity requires a 4.x browser. IE 5.0, Safari, and Mozilla supported.
This page was written in Javascript.
Extensively rewritten from 12/15/2000-12/19/2000 to isolate javascript Oligo object behaviors for teaching purposes.
This page may be freely distributed for any educational or non-commercial use.

About the Calculations

Thermodynamic Calculations

The nearest neighbor and thermodynamic calculations are done essentially as described by Breslauer *et al.*, *Proc. Nat. Acad. Sci.* **83**, 3746-50, 1986 (Abstract) but using the values published by Sugimoto *et al.*, *Nucl. Acids Res.* **24**, 4501-4505, 1996 (Abstract). This program assumes that the sequences are not symmetric and contain at least one G or C. The minimum length for the query sequence is 8.

The melting temperature calculations are based on the simple thermodynamic relationship between entropy, enthalpy, free energy and temperature, where

h b c e b g c ch

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Enter Oligonucleotide Sequence Below
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Nucleotide base codes

CCG GAG GCG TAA GAG TTC CTC GGC TCG GTC GGG CTT GCC CCT

Reverse Complement Strand(5' to 3') is:

AGG GGC AAG CCC GAC CGA GCC GAG GAA CTC TTA CGC CTC CGG

Number of Fluorescent tags per strand:

0 6-FAM 0 TET 0 HEX 0 TAMRA DNA

Minimum base pairs required for single primer self-dimerization: 5

Minimum base pairs required for a hairpin : 4

Calculate | SWAP STRANDS | BLAST2 | Check Self-Complementarity

Physical Constants

Length: 42 bases

GC content: 69 %

Molecular Weight: 13056.3 4

1 ml of a sol'n with an Absorbance of 1 at 260 nm

is 2.408 microMolar 5 and contains 31.4 micrograms.

Melting Temperature (T_M) Calculations

1 77 °C (Basic)

2 76 °C (Salt Adjusted)

3 76 °C (Nearest Neighbor)

50 nM Primer

50 mM Salt (Na^+)

Thermodynamic Constants
Conditions: 1 M NaCl at 25°C at pH 7.

RlogK 33.404 cal/(°K*mol)

deltaH 386.9 Kcal/mol

deltaG 71.5 Kcal/mol

deltaS 1000.4 cal/(°K*mol)

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or Internet Explorer version 3.0 or later, or another Javascript-capable browser
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The melting temperature calculations are based on the simple thermodynamic relationship between entropy, enthalpy, free energy and temperature, where

h b c e b g c ch

$$\Delta H = \Delta G + T\Delta S$$

The change in entropy (order or a measure of the randomness of the oligonucleotide) and enthalpy (heat released or absorbed by the oligonucleotide) are directly calculated by summing the values for nucleotide pairs obtained by Breslauer *et al.*, *Proc. Nat. Acad. Sci.* **83**, 3746-50, 1986. The relationship between the free energy and the concentration of reactants and products at equilibrium is given by

$$\Delta G = RT \ln\left(\frac{[DNA \bullet primer]}{[DNA][primer]}\right)$$

Substituting the two equations gives us

$$\Delta H = T\Delta S + RT \ln\left(\frac{[DNA \bullet primer]}{[DNA][primer]}\right)$$

and solving for temperature T gives

$$T = \frac{\Delta H}{\Delta S + R \ln\left(\frac{[DNA \bullet primer]}{[DNA][primer]}\right)}$$

We can assume that the concentration of DNA and the concentration of the DNA-primer complex are equal, so this simplifies the equation considerably. It has been determined empirically that there is a 5 (3.4 by Sugimoto et al.) kcal free energy change during the transition from single stranded to B-form DNA. This is presumably a helix initiation energy. Finally, adding an adjustment for salt gives the equation that the Oligo Calculator uses:

$$T = \frac{\Delta H - 5\frac{kcal}{^\circ K\ mole}}{\Delta S + R \ln\left(\frac{1}{[primer]}\right)} + 16.6 \log_{10}([Na^+])$$

No adjustment constant for salt concentration is needed, since the various parameters were determined at 1 Molar NaCl, and the log of 1 is zero.

ASSUMPTIONS:
The thermodynamic calculations assume that the annealing occurs at pH 7.0. The melting temperature (Tm) calculations assume the sequences are not symmetric and contain at least one G or C. The oligonucleotide sequence should be at least 8 bases long to give reasonable Tms.

Basic Melting Temperature (Tm) Calculations

h b c e b g c c h

The two standard approximation calculations are used. For sequences less than 14 nucleotides the formula is

$$Tm = (wA+xT) * 2 + (yG+zC) * 4$$

where w,x,y,z are the number of the bases A,T,G,C in the sequence, respectively.

For sequences longer than 13 nucleotides, the equation used is

$$Tm = 64.9 + 41*(yG+zC-16.4)/(wA+xT+yG+zC)$$

ASSUMPTIONS:
Both equations assume that the annealing occurs under the standard conditions of 50 nM primer, 50 mM Na^+, and pH 7.0.

Salt Adjusted Melting Temperature (Tm) Calculations

A variation on two standard approximation calculations are used. For sequences less than 14 nucleotides the same formula as the basic calculation is use, with a salt concentration adjustment

$$Tm = (wA+xT)*2 + (yG+zC)*4 - 16.6*\log_{10}(0.050) + 16.6*\log_{10}([Na^+])$$

where w,x,y,z are the number of the bases A,T,G,C in the sequence, respectively.

The term $16.6*\log_{10}([Na^+])$ adjusts the Tm for changes in the salt concentration, and the term $\log_{10}(0.050)$ adjusts for the salt adjustment at 50 mM Na^+. Other monovalent and divalent salts will have an effect on the Tm of the oligonucleotide, but sodium ions are much more effective at forming salt bridges between DNA strands and therefore have the greatest effect in stabilizing double-stranded DNA.

For sequences longer than 13 nucleotides, the equation used is

$$Tm = 81.5 + (41 * (yG+zC)/(wA+xT+yG+zC)) - (500/(wA+xT+yG+zC)) + 16.6*\log_{10}([Na^+]) - 0.62F$$

This equation is most accurate for sequences longer than 50 nucleotides. It is valid for oligos longer than 50 nucleotides from pH 5 to 9. Symbols and salt adjustment term as above, with the term $(41 * (yG + zC-16.4)/(wA + xT + yG + zC))$ adjusting for G/C content and the term $(500/(wA + xT + yG + zC))$ adjusting for the length of the sequence, and F is the percent concentration of formamide.

For more information please see the reference:
Howley, P.M; Israel, M.F.; Law, M-F.; and M.A. Martin "A rapid method for detecting and mapping homology between heterologous DNAs. Evaluation of polyomavirus genomes." *J. Biol. Chem.* **254**, 4876-4883, 1979.

RNA melting temperatures

$$Tm = 79.8 + 18.5*\log_{10}([Na^+]) + (58.4 * (yG+zC)/(wA+xT+yG+zC)) + (11.8 * ((yG+zC)/(wA+xT+yG+zC))^2) - (820/(wA+xT+yG+zC))$$

Where yG+zC are the mole fractions of G and C in the oligo, L is the length of the shortest strand in the duplex.

ASSUMPTIONS:
These equations assume that the annealing occurs under the standard conditions of 50 nM primer and pH 7.0.

Molecular Weight Calculations

DNA Molecular Weight (for instance Oligonucleotides)
Molecular Weight = $(A_n \times 313.21) + (T_n \times 304.2) + (C_n \times 289.18) + (G_n \times 329.21) + 79.0$
A_n, T_n, C_n, and G_n are the number of each respective nucleotide within the polynucleotide. The addition of 79.0 gm/mole to the molecular weight takes into account the 5' monophosphate left by most restriction enzymes. No phosphate is present at the 5' end of strands made by primer extension.

h b c e b g c c h

RNA Molecular Weight (for instance from an RNA transcript)
Molecular Weight = (A_n x 329.21) + (U_n x 306.17) + (C_n x 305.18) + (G_n x 345.21) + 159.0
A_n, U_n, C_n, and G_n are the number of each respective nucleotide within the polynucleotide. Addition of 159.0 gm/mole to the molecular weight takes into account the 5' triphosphate.

OD Calculations

Molar Absorptivity values in 1/(Moles cm)

Residue	Moles^{-1} cm^{-1}	A_{max}(nm)	Molecular Weight (after protecting groups are removed)
Adenine (dAMP, Na salt)	15200	259	313.21
Guanine (dGMP, Na salt)	12010	253	329.21
Cytosine (dCMP, Na salt)	7050	271	289.18
Thymidine (dTMP, Na salt)	8400	267	304.2
RNA nucleotides			
Adenine (AMP, Na salt)	15400	259	329.21
Guanine (GMP, Na salt)	13700	253	345.21
Cytosine (CMP, Na salt)	9000	271	305.18
Uradine (UMP, Na salt)	10000	262	306.2
Other nucleotides			
6' FAM	20960		537.46
TET	16255		675.24
HEX	31580		744.13
TAMRA	31980		

Assume 1 OD of a standard 1ml solution, measured in a cuvette with a 1 cm pathlength.

6-FAM:

Chemical name: **6-carboxyfluorescein**
Absorption wavelength maximum: **495 nm**
Emission wavelength maximum: **521 nm**
Molar Absorptivity at 260nm: **20960** Moles^{-1} cm^{-1}

TET:

Chemical name: **4, 7, 2', 7'-Tetrachloro-6-carboxyfluorescein**
Absorption wavelength maximum: **519 nm**
Emission wavelength maximum: **539 nm**
Molar Absorptivity at 260nm: **16255** Moles^{-1} cm^{-1}

h b c e b g c c h

HEX:

Chemical name:	**4, 7, 2', 4', 5', 7'-Hexachloro-6-carboxyfluorescein**
Absorption wavelength maximum:	**537 nm**
Emission wavelength maximum:	**556 nm**
Molar Absorptivity at 260nm:	**31580** Moles^{-1} cm^{-1}

TAMRA:

Chemical name:	**N, N, N', N'-tetramethyl-6-carboxyrhodamine**
Absorption wavelength maximum:	**555 nm**
Emission wavelength maximum:	**580 nm**
Molar Absorptivity at 260nm:	**31980** Moles^{-1} cm^{-1}

Nucleotide base codes (IUPAC)

Symbol:	nucleotide(s)		
A	adenine	**M** A or C	**K** G or T
C	cytosine	**R** A or G	**V** A or C or G; not T
G	guanine	**W** A or T	**H** A or C or T; not G
T	thymine in DNA; uracil in RNA	**S** C or G	**D** A or G or T; not C
		Y C or T	**B** C or G or T; not A
N	A or C or G or T		

Most recent version is available at URL: http://www.basic.northwestern.edu/biotools/oligocalc.html

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The current version is the result of efforts by the following people:

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h b c e b g c c h

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Uppercase/lowercase strand complementation problem described by Alexey Merz alexey@dartmouth.edu

Oligo Calculator version 3.03 (last modified by WAKibbe 02/12/2004)

h b c e b g c ch

Alignment of SEQ ID NO: 1

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  AUTHORS   Tikhonov,A.P., SanMiguel,P.J., Nakajima,Y., Gorenstein,N.M.,
            Bennetzen,J.L. and Avramova,Z.
  TITLE     Colinearity and its exceptions in orthologous adh regions of maize
            and sorghum
  JOURNAL   Proc. Natl. Acad. Sci. U.S.A. 96 (13), 7409-7414 (1999)
   PUBMED   10377428
REFERENCE   2  (bases 1 to 160480)
  AUTHORS   SanMiguel,P.J., Tikhonov,A. and Bennetzen,J.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (25-JAN-1999) Biological Sciences, Purdue University,
            Hansen LSRB, Rm. 339, West Lafayette, IN 47907, USA
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ORIGIN
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June 22, 1999

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Db    57404 CCTCGGCTCAGTCGGCCTCGCCACT 57428
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